AD_____

Award Number: DAMD17-99-1-9099

TITLE: Peptide Antiestrogens for Human Breast Cancer Therapy

PRINCIPAL INVESTIGATOR: Richard Pietras, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of California, Los Angeles
Los Angeles, California 90095-1406

REPORT DATE: July 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2000	Annual (1 Jul 99 - 30 Jun 00)

4. TITLE AND SUBTITLE
Peptide Antiestrogens for Human Breast Cancer Therapy

5. FUNDING NUMBERS
DAMD17-99-1-9099

6. AUTHOR(S)
Richard Pietras, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of California, Los Angeles
Los Angeles, California 90095-1406

E-MAIL:
rpietras@ucla.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Growth of breast cells is regulated by estrogens. This hormone binds receptors present in 60-70% of breast cancers. Estrogen receptor α (ER) is a phosphoprotein that regulates transcription and growth by binding to estrogen response elements (ERE) in DNA. Inactive ER is a monomer that forms a dimer on estrogen-induced phosphorylation. This signaling complex recruits steroid receptor coactivator proteins, such as SRC-1, that are essential for growth. Transcriptional activity of ER is regulated by distinct conformational states resulting from ligand binding. Peptides derived from the interacting sites of ER proteins may selectively inhibit ER signaling and function as antiestrogens. To test this hypothesis, we synthesized small peptides that mimic a highly conserved ER sequence including tyrosine-537 and surrounding leucine residues. Peptide antiestrogens, but not control peptides, blocked association of ER with SRC-1, and, in gel mobility shift assays, disrupted binding of ER to (^{32}P)-ERE. Using *in vitro* studies with breast cells, treatment with estradiol stimulated cell growth 4-fold by 72 h. This effect of estrogen was blocked by pre-treatment with 10^{-9} M peptide antiestrogens conjugated with Antennapedia carrier but not by control peptides. In *in vivo* tumor xenograft experiments, treatment of nude mice with peptide antiestrogens showed significant activity in arresting growth of estrogen-dependent breast tumors.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer ,estrogen receptor, antiestrogen, peptide	10
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

___ Where copyrighted material is quoted, permission has been obtained to use such material.

___ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

___ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Richard J. Dietrich MD 6/28/00

PI - Signature Date

Table of Contents

INTRODUCTION

The estrogen receptor (ER) is a phosphoprotein found in 60-70% of human breast tumors at diagnosis (1-3). Antiestrogen therapy with tamoxifen, a partial agonist to estrogen receptor, has had a significant impact on survival in patients with breast cancer, but tamoxifen also has several undesirable side-effects. The antitumor efficacy of antiestrogen therapy is clearly due to close regulation of breast cell growth by estrogens. However, as breast cancer progresses, it becomes resistant to estrogens, and most patients no longer respond to current antiestrogens (1-3). The continued expression of estrogen and/or progesterone receptors in most patients with tumor progression on tamoxifen indicates that mechanisms for resistance other than receptor loss are common in breast cancer and are responsible for treatment failure (4,5).

This proposal is based on new understanding of the biology of estrogen receptor, a phosphoprotein that forms a dimer required for binding to specific estrogen response elements in the nucleus, leading to promotion of breast cancer cell growth (6-8). Transcriptional activity of ER is now known to be related to the conformational state of the receptor, especially with respect to the molecular orientation of helix-12 in the ligand-binding domain of ER (8). Helix-12 contains leucine-rich regions that interact with steroid receptor coactivator proteins that, in turn, regulate transcription (9,10) (see Table 1).

TABLE 1. a) Amino acid sequence comparison of AF2 α-helix region in ER from several species, with helix-12 starting at residue 538. Conserved region is in boldface. b) Sequence comparison with ER-ß. c) Sequence alignment of nuclear receptor proteins, including progesterone and androgen receptors (9,11).

a	**PL-YDLLLEML-DA**	535-546	human ER
	PL-YDLLLEML-DA	539-550	mouse ER
	PL-YDLLLEML-DA	527-538	xenopus ER
b	**PV-YDLLLEML-NA**	433-444	human ER-ß
c	EF-PEMMSEVI-AA	904-915	progesterone receptor
	DF-PEMMAEI I-SV	889-900	androgen receptor

Manipulation of helix-12 interactions with coactivator proteins may provide alternate approaches to antihormone therapy. We have synthesized peptides designed to disrupt binding of estrogen receptor with coactivator proteins. Our specific aims for this project include the following experimental objectives:

1) Synthesis of small phosphotyrosyl-peptides targeted to a highly conserved sequence in estrogen receptor including tyrosine-537 and surrounding leucine residues. Experiments are planned to evaluate the efficacy of these peptide antiestrogens in antagonizing estrogen receptor activity in breast cancer cells, including blockade of estrogen receptor dimerization, reduction of estrogen receptor association with steroid receptor coactivator protein and suppression of estrogen receptor binding to specific estrogen-response elements in DNA.

2) Evaluation of the antitumor efficacy *in vitro* and *in vivo* of small phosphotyrosyl- and malonyltyrosyl-peptides that suppress dimerization and DNA binding of estrogen receptor in human breast cancer cells. Alternative modes for the efficient delivery of low concentrations of peptides *in vivo* will be considered, and effects of peptide antiestrogens on bone, serum cholesterol, uterus and body composition will be evaluated in rodent models.

It is important to develop new antiestrogens which work through different mechanisms of interaction with ER, since these would likely prove useful in treatment of breast cancers that become resistant to conventional antiestrogens. This project offers an innovative approach to antitumor therapy with the potential for developing

novel antiestrogens with minimal toxicity to noncancerous tissues, and it may further advance our understanding of the role of estrogen receptor in hormone action.

BODY: RESEARCH PROGRESS

A full report on research progress on this proposal was presented in a platform session at the Era of Hope Meeting in Atlanta, June 10, 2000 (12).

AIM 1) *Synthesis of small phosphotyrosyl-peptides targeted to tyrosine-537 and the neighboring leucine-rich region in ER and evaluation of their efficacy in the blockade of ER dimerization and ER binding to steroid receptor coactivator and DNA in human breast cancer.*

Peptides disrupt binding of ER with DNA

In order to evaluate potential antiestrogen effects of peptides that mimic the initial sequence in helix-12 in ER, peptides were synthesized by established methods with N-terminal acetylation and a C-terminal amide in the UCLA/Jonsson Cancer Center Peptide Synthesis Facility (12,13). Peptide constructs were characterized by HPLC and mass spectral analysis and found to be > 95% pure. The octapeptide, pY8, contains the sequence:

N-Pro-Leu-*pTyr-Asp-Leu-Leu-Leu-Glu-C (PLpYDLLLE)

and its nonphosphorylated analog, conY8, has the sequence :

N-Pro-Leu-Tyr-Asp-Leu-Leu-Leu-Glu-C (PLYDLLLE).

An additional control peptide with a scrambled sequence, con8, is shown below:

N-Val-Pro-Leu-Asp-Leu-Leu-Leu-Glu-C (VPLDLLLY).

Other peptides of varying size (5-mer and 12-mer) to ascertain the optimal preparation for use in cellular studies have also been prepared (refer to Table 2 in original proposal) (12).

Interaction of ER with nuclear ERE is prerequisite for activation of transcription. To assess specific binding of ER with ERE, we used purified recombinant human ER from MCF-7 breast cancer cells (13). A double-stranded 27-bp probe [5'-GATCCTAGAGGTCACAGTGACCTACGA-3'] encoding the Xenopus vitellogenin A_2 ERE was ^{32}P-end-labeled with polynucleotide kinase. Gel mobility shift assays for the human ER were performed as described (13). The ER in 20 mM reaction buffer (HEPES, pH 7.5, 1 mM EDTA, 100 mM Kcl, 1 mg/ml BSA, 100 nM estradiol, 15% glycerol, and proteinase inhibitors) was incubated with 500 ng of poly (dI-dC) for 15 min at 4 C, and then 20 fmol of the ^{32}P-labeled ERE probe was added for 15 min at 4 C in a total volume of 20 µl. Samples were loaded onto a pre-electrophoresed 5% polyacrylamide gel followed by electrophoresis with cooling at 175 V for 3h in 25 mM TRIS, pH 8.0 with 152 mM glycine and 1 mM EDTA. Under these conditions, the human ER reacts specifically with synthetic ERE in the gel mobility shift assay, allowing formation of an ER-ERE complex (13) (FIG. 1).

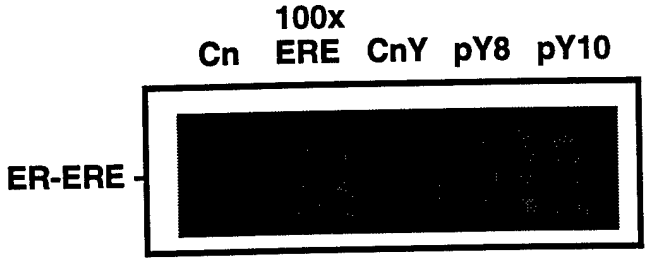

FIG. 1. Gel mobility shift assays of human ER and estrogen-responsive element (ERE). Purified human ER (60 nM) incubated with control solution (Cn), 100-fold molar excess of unlabelled ERE (100x ERE) or with peptides at 2.5 µM. ER was incubated with peptides for 15 min at 4°C, then with 100 nM estradiol-17ß and

^{32}P-ERE. Peptides included peptide pY8, control Y8 peptide (CnY), and a decapeptide, N-Val-Pro-Leu-pTyr -Asp-Leu-Leu-Leu-Glu-Met-C (pY10).

This ER-ERE interaction is blocked by competition with 2.5 μM pY8-peptide but not by competition with 5 μM conY8-peptide (43) or con8-peptide (data not shown). Thus, peptide antiestrogens disrupt ER binding to a specific ERE *in vitro*. However, the IC_{50} for this effect may exceed by 100-fold that required for other cellular actions of the peptides (see ER/SRC-1 interaction below). Further work on the nature and significance of this peptide effect is planned.

Peptides interfere with ER dimerization

Experiments to assess effects of peptides on ER dimerization, using molecular sizing chromatography with Sephadex G-200 (13,14), are also underway. Using this approach, we find that 25 μM pY8 inhibits dimerization of ER (12,13). As with peptide inhibition of ER binding to DNA, peptide interference with ER dimerization appears to occur at a higher IC_{50} than other cellular actions of the peptide (see ER/SRC-1 interaction below). We plan to confirm these findings in the next grant year.

Peptides block molecular association between ER and steroid receptor coactivator proteins

As noted above, upon activation *in vivo*, ER bind to DNA response elements and recruit co-activator proteins and general transcription factors to form an active complex for stimulation of gene expression. Steroid receptor coactivator-1 (SRC-1) is a well-characterized coactivator protein (165 kd) that mediates steroid hormone responses by promoting receptor-dependent transactivation of genes (15,16), and disruption of the SRC-1 gene results in partial resistance to hormone (16). Short sequence motifs in SRC-1 and other coactivators are necessary to mediate the binding of these proteins to nuclear receptors (10). In order to assess the effect of peptide antiestrogens on the interaction between ER and SRC-1, T47D breast cancer cells were treated *in vitro* with or without 1nM estradiol-17ß, and cell lysates were prepared for immunoprecipitation with antibody to ER, followed by gel electrophoresis and immunoblotting with antibody to SRC-1 as before (17). In the absence of peptide antiestrogens, SRC-1 and ER form a binding complex beginning at 15 min after estrogen treatment, and the association is maximal by 30 min. Prior incubation of breast cells with pY8 interferes with this ER/SRC-1 binding (FIG. 2). In contrast, pre-treatment of T47D breast cancer cells with conY8 or con8 elicits no effect on ER/SRC-1 binding *in vitro* (FIG. 2) (12).

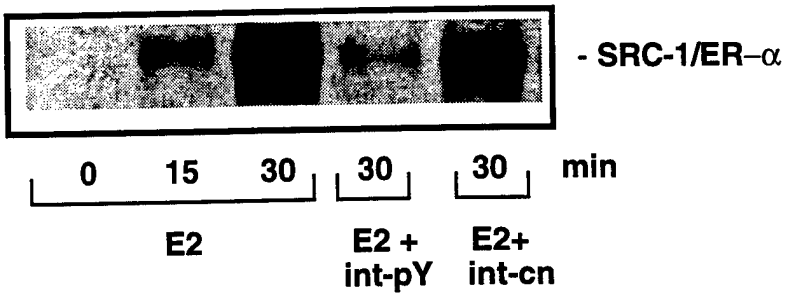

FIG. 2. Effect of estradiol-17ß (E2) on the association of ER and SRC-1 in T47D cells. T47D breast cancer cells were treated *in vitro* with 10nM E2 or control vehicle for 15-30 min. For more efficient delivery of peptide, pY8 was coupled with a short peptide from the homeodomain of antennapedia, a vector that promotes internalization of peptides linked to its carboxy-terminus (int-pY8) (18). Int-pY8 or int-peptide control (int-cn) were added 30 min before E2 for an additional 30 min. Lysates were prepared and processed as before. Samples were immunoprecipitated with monoclonal anti-ER antibody-10 (Neomarker), followed by electrophoresis and immunoblot with monoclonal anti-SRC-1 antibody (Affinity Bioreagents).

Thus, tyrosine-537 and adjacent leucine residues in ER may be important in regulating ER/SRC-1 interaction in breast cancer. In contrast to peptide effects on ER dimerization and DNA binding *in vitro*, the peptide effect on ER/SRC-1 association can be elicited at nanomolar concentrations of active peptides. Thus, peptide blockade of the binding of receptor coactivator protein with ER may have more physiologic significance *in vivo*.

AIM 2) *Evaluation of the antitumor efficacy in vitro and in vivo of phosphotyrosyl- and malonyltyrosyl-peptides that suppress biologic activity of ER in human breast cancers*

Peptide antitumor effects *in vitro* and *in vivo*

One problem with use of phosphotyrosyl-peptides *in vitro* or *in vivo* is susceptibility of the constructs to degradation by cellular tyrosine phosphatase enzymes. To address this difficulty, we prepared phosphotyrosyl-mimic peptides that use malonic acid rather than phosphate residues at tyrosine sites. Malonyl-tyrosine residues appear to mimic the phosphotyrosine conformation in proteins and evade the action of cellular enzymes targeted to phosphotyrosine (19,20). The malonyltyrosyl-peptides contain the sequence surrounding tyrosine-537 in ER (12,13) (see Table 1). Malonyltyrosyl-octapeptide, mY8, was synthesized by established methods and contains the sequence:

N-Pro-Leu-mTyr-Asp-Leu-Leu-Leu-Glu-C (PLmYDLLLE).

We find that malonyltyrosyl-peptide constructs, as phosphotyrosyl-peptides, suppress binding of ER to specific ERE in human breast cancer cells. This ER-ERE interaction is blocked by competition with 2.5 μM mY8-peptide but not by 5 μM conY8-peptide (12,13). Further evaluation of the efficacy of 5- and 12-mer malonyltyrosyl-peptides are planned to find the optimal peptide sequence for use in *in vivo* studies.

Using the pY8-internalization vector (int-pY8), we find that nanomolar concentrations of the peptide have good efficacy in disruption of estrogen-induced growth of human breast cancer cells (12). The anticipated growth stimulation by estrogen is found after treatment of MCF-7 cells with control internalization peptide alone, exceeding growth of control cells in the absence of estrogen by 3-fold. Similarly, a low concentration of free pY8-peptide alone in solution (25 μM) does not alter the growth response to estrogen. However, peptide antiestrogen coupled with internalization peptide does suppress the expected growth effect of estrogen ($P<0.001$). A dose-response study using concentrations of drug ranging from 0.02 to 500 nM shows that the pY8-internalization peptide is effective in growth inhibition of MCF-7 breast cancer cells at concentrations < 25 nM (12). Studies of breast tumor xenografts *in vivo* are also underway using methods as before (17) with peptide delivery by intraperitoneal injection (13,17).

We plan to study the biologic activity of other peptide antiestrogens using identical assays for cell proliferation (12,13)(see original proposal). Our goal in these studies will be to determine minimum peptide sequence required for specificity and efficacy. Evaluation of the cellular uptake of peptides with and without internalization vector or liposomes is being done using established fluorescent-labeling techniques (12,13). Depending on results of these studies, additional peptides may be designed and synthesized for testing. As reported before, tamoxifen completely suppresses the growth of MCF-7 cells, but tamoxifen elicits only marginal inhibition of the growth of ZR-75-1 cells and, acting as a partial agonist to ER, the drug appears to stimulate the growth of T47D breast cancer cells (5,21,22). Comparative cell growth studies with the latter cell types, as well as with BT-20 and SKBR3 breast cancer cells with no detectable wild-type ER and tamoxifen-resistant MCF-7 cells with HER-2/neu tyrosine kinase overexpression (17) are also planned.

Estrogen is a key regulatory hormone which, in addition to its role in reproductive tissues, affects a number of physiological systems, including the skeleton and cardiovascular system. In order to evaluate tissue selectivity of the final peptide formulations, these agents will be given to immature female mice for 3 days, and effects on uterine wet and dry weight will be assessed (23). In addition, uterine hypertrophy will be evaluated in older (up to 15-mo-old) female mice treated with peptides for 28-90 days. Effects of peptides on total serum cholesterol, fat body mass and lean body mass in these aged female rodents will be tested. In ovariectomized female mice, we plan to assess effects of peptides on ovariectomy-induced increments in body weight gain, total serum cholesterol, and bone loss. Following ovariectomy, rodents will be assigned to control groups including both placebo and positive control with estrogen replacement using established methods (23). Our

goal is to develop novel antitumor agents with minimal toxicity to noncancerous tissues, and these experiments will help to establish the response profile and tissue selectivity of peptide antiestrogens.

KEY RESEARCH ACCOMPLISHMENTS

- Small leucine-rich peptides that mimic ER sequence at the start of helix-12 reduce the formation of ER homodimers and reduce binding of ER to ERE.
- Small leucine-rich peptides that mimic ER sequence at the start of helix-12 suppress association of ER with SRC-1.
- Estrogen-dependent growth of human breast cancer cells is blocked by pre-treatment with small leucine-rich peptides that mimic ER sequence at the start of helix-12.

REPORTABLE OUTCOMES

Presentations

1. "Small Molecule Inhibitors of Estrogen Receptor Function". Presented at Molecular Oncology Seminar, Genentech, South San Francisco (1999).
2. "Peptide antagonists of the estrogen receptor block growth of human breast cancer cells". Presented at Era of Hope Department of Defense Breast Cancer Research Program Meeting, Atlanta (2000).

Abstracts

1. Pietras, R.J., Marquez, D., Chen, X. and Li, D. (2000). Peptide antagonists of the estrogen receptor block growth of human breast cancer cells. Era of Hope DOD Breast Cancer Research Program Proceedings, **2**: 535.

No manuscripts, patents, degrees, development of cell lines, informatics or additional funding or research opportunities to be reported at this time.

CONCLUSIONS

This project is a new approach to antitumor therapy with the potential for developing antiestrogen treatments with minimal toxicity to noncancerous tissues. Small leucine-rich peptides that mimic ER sequence at the start of helix-12 in the receptor molecule are especially effective in suppressing the association of ER with SRC-1. This molecular action appears to elicit blockade of breast cancer cell proliferation. The results of our preliminary studies suggest that treatment with small peptide antiestrogens may prove more effective than drugs currently available in blocking the growth-promoting signals of estrogen receptors. Further studies to assess the optimal constructs and the safety of these peptide compounds are planned. This work provides good evidence of target validation for helix-12 in estrogen receptor.

REFERENCES

1). Harris J, M Lippman, U Veronesi and W Willett (1992). Breast cancer. New Engl. J. Med., **327** : 473-480.
2.) Katzenellenbogen BS, M Montano, K Ekena, M Herman and E McInerney (1997). Antiestrogens: Mechanisms of action and resistance in breast cancer. Breast Cancer Res. Trtmt.,**44** : 23-38.

3.) Tonetti D and VC Jordan (1997). The role of estrogen receptor mutations in tamoxifen-stimulated breast cancer. J. Steroid Biochem. Mol. Biol., **62**: 119-128.
4.) Encarnacion C, D Ciocca, W McGuire, G Clark, S Fuqua and C Osborne (1993). Measurement of steroid hormone receptors in breast cancer patients on tamoxifen. Breast Cancer Res. Trtmt., **26**: 237-246.
5.) Mullick A and P Chambon (1990). Characterization of the estrogen receptor in two antiestrogen-resistant cell lines, LY2 and T47D. Cancer Res. **50**: 333-338.
6.) Magliaccio A, A Rotondi and F Auricchio (1986). Estradiol receptor: phosphorylation on tyrosine in uterus and interaction with anti-phosphotyrosine antibody. EMBO J., **5** : 2867-2872.
7.) Brandt M. and L. Vickery (1997). Cooperativity and dimerization of recombinant human estrogen receptor hormone-binding domain. J. Biol. Chem., 272: 4843-4849.
8.) Brzozowski A., A. Pike, Z. Dauter, R. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G. Greene, J. Gustafsson and M. Carlquist (1997). Molecular basis of agonism and antagonism in the oestrogen receptor. Nature, **389**: 753-758.
9.) Danielian P, R White, L Lees and M Parker (1992). Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. EMBO J., **11**: 1025-1033.
10.) Heery D., E. Kalkhoven, S. Hoare and M. Parker (1997). A signature motif in transcriptional co-activators mediates binding to nuclear receptors. Nature, 387: 733-736.
11.) White R, M Sjoberg, E Kalkhoven and MG Parker (1997). Ligand-independent activation of the oestrogen receptor by mutation of a conserved tyrosine. EMBO J., **16** : 1427-1435.
12.) Pietras RJ, D Marquez, X Chen and D Li (2000). Peptide antagonists of the estrogen receptor block growth of human breast cancer cells. Era of Hope DOD Breast Cancer Research Program Proceedings, **2** : 535.
13.) Pietras RJ and J Lee (1997). Antitumor effect of phosphotyrosyl-peptides that block binding of estrogen receptor to DNA in human breast cancer cells. Proc.Am. Assoc. Cancer Res., **38**: 174.
14.) Pietras RJ and CM Szego (1980). Partial purification and characterization of oestrogen receptors in subfractions of hepatocyte plasma membrane. Biochem. J., **191** : 743-760.
15.) Onate S., V. Boonyaratanakornkit, T. Spencer, S. Tsai, M.-J. Tsai, D. Edwards and B. O'Malley (1998). The steroid receptor coactivator-1 contains multiple receptor interacting and activation domains that cooperatively enhance the activation function 1 (AF1) and AF2 domains of steroid receptors. J. Biol. Chem., **273**: 12101-12108.
16.) Xu J., Y. Qiu, F. DeMayo, S. Tsai, M.-J. Tsai and B. O'Malley (1998). Partial hormone resistance in mice with disruption of the steroid receptor coactivator-1 (SRC-1) gene. Science, **279**: 1922-1925.
17.) Pietras RJ, J Arboleda, D Reese, N Wongvipat, M Pegram, L Ramos, C Gorman, M Parker, M Sliwkowski, and D Slamon (1995). HER-2 tyrosine kinase pathway targets estrogen receptor and promotes hormone-independent growth in human breast cancer cells. Oncogene, **10** : 2435-2446.
18.) Bonfanti M, S Taverna, M Salmona, M D'Incalci and M Broggini (1997). p21WAF1-derived peptides linked to an internalization peptide inhibit human cancer cell growth. Cancer Research, **57** : 1442-1446.
19.) Kole H, M Akamatsu, B Ye, X Yan, D Barford, P Roller and T Burke, Jr. (1995). Protein tyrosine phosphatase inhibition by a peptide containing the phosphotyrosyl mimetic L-O-malonyltyrosine. Biochem. Biophys. Res. Commun., **209**: 817-822.
20.) Ye B, M Akamatsu, S Shoelson, G Wolf, S Giorgetti-Peraldi, X Yan, P Roller and T Burke, Jr. (1995). L-O-(2-Malonyl)tyrosine: a new phosphotyrosyl mimetic for the preparation of src homology 2 domain inhibitory peptides. J Med Chem, **38** : 4270-4275.
21.) Coradini D, A Biffi, V Cappelletti and G Di Fronzo (1995). Influence of different combinations of tamoxifen and toremifene on estrogen receptor-positive breast cancer cell lines. Cancer Detection Prevention, **19**: 348-354.
22.) Couillard S, M Gutman, C Labrie, A Belanger, B Candas and F Labrie (1998). Comparison of the effects of the antiestrogens EM-800 and tamoxifen on the growth of human breast ZR-75-1 cancer xenografts in nude mice. Cancer Res.,**58**: 60-64.
23.) Ke H, V Paralkar, W Grasser, D Crawford, H Qi, H Simmons, C Pirie, K Chidsey-Frink, T Owen, S Smock et al. (1998). Effects of CP-336,156, a new nonsteroidal estrogen agonist/antagonist, on bone, serum cholesterol, uterus and body composition in rat models. Endocrinology, **139**: 2068-2076.